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THE ZOÖCHLORELLÆ OF *FRONTONIA LEUCAS.*

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INTRODUCTION.

It is common knowledge that many organisms of the animal kingdom harbor within their bodies various forms of algæ. Dangeard ('02) writes at length on the probable relation of the zoöchlorellæ, in *Paramecium bursaria*, to their host. The experiments of Lipska ('10) on a culture of *Paramecium caudatum* which contained green algæ are also worthy of note. Geza Entz ('81–2) presents evidence which he thinks is indicative of a symbiotic relation between *Stentor polymorphus* and green algæ. Bary ('79) was the first investigator to allude to the relation between the zoöchlorellæ and their host as a symbiotic one. But Dangeard ('02) says that the relation is incompletely proven, and Bouvier adds that we have not a definite example demonstrating the usefulness of the algæ to their host.

The work of this paper was conducted in an effort to add further knowledge on the relation of endoplasmic algæ to their host. The host used in these experiments has been identified as *Frontonia leucas* Ehrenberg.

I wish to express my gratitude to Dr. B. D. Reynolds, under whose direction this work was done, for reading the manuscript and making a number of important suggestions. I am also indebted to Dr. W. A. Kepner and Mr. J. B. Looper for helpful advice and criticisms.

DISAPPEARANCE OF ZOÖCHLORELLÆ FROM HOST.

In *Frontonia* taken from a collection made near the University of Virginia, from a very small stagnant pool, on June 23, 1925,

the zoöchlorellæ were innumerable. Artificial cultures were attempted with little success. For this reason collections were kept in the laboratory just as they were when taken from the pool, except that fresh spring water was added daily.

After thirty days specimens from these cultures showed a decided decrease in the number of zoöchlorellæ. Fresh collections were then made and treated as before with like results. It was found that with such treatment *Frontonia* would, in from thirty to forty days, be partially if not entirely free of zoöchlorellæ.

On September 15, 1925, seven cultures in fresh spring water [1] were started, using Syracuse watch glasses as containers. Twelve specimens were placed in each culture, and fresh spring water was added daily. By September 18 approximately fifty per

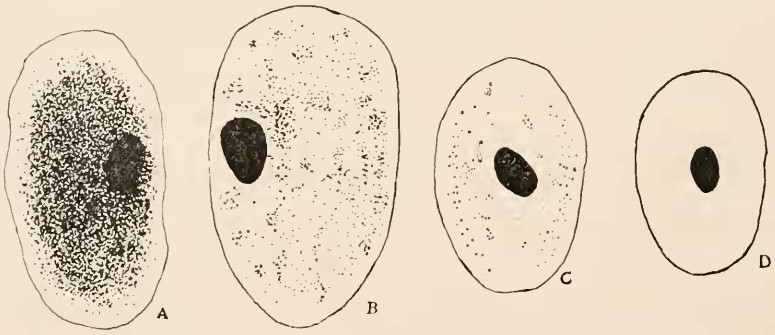

FIG. 1. Camera lucida drawings. × 375. *A*, normal specimen taken on date of collection. *B*, after twelve days in fresh spring water. *C*, after twenty-five days in fresh spring water. *D*, after forty days in fresh spring water.

cent. of the total number of the specimens had disappeared. September 21, all specimens had disappeared from five cultures.

[1] Analysis of the spring water was made by Mr. R. K. Witt, under the direction of Dr. John H. Yoe of the Department of Chemistry, University of Virginia, and showed the following:

	Parts Per Million.
Total solids	37.6
Fixed residue	28.0
Loss on ignition	9.6
Organic matter	Very small amount as indicated by a slight charring on ignition.

The pH value of the spring water was fairly constant at 7.2.

Three living specimens were found in one, and five in the other. On September 22 duplicate cultures were started with similar results.

September 30, collections of *Frontonia* harboring innumerable zoöchlorellæ were made and change from the original culture medium to fresh spring water was made gradually by drawing off a part of the medium daily and replacing it with fresh spring water. Under these conditions it was observed that within forty days the zoöchlorellæ had completely disappeared, while the ciliates showed no signs of death.

Slides made at intervals extending over the forty-day period corroborate these observations (Fig. 1). Various stains and fixatives were used in making the slides, but the most satisfactory results were obtained from Looper's fixative [1] and iron hæmotoxlyn.

From these observations it is evident that *Frontonia* may be freed from their zoöchlorellæ by a gradual transference from their natural habitat to a medium of fresh spring water.

Increase in Number of Zoöchlorellæ in Host.

In a collection made from a large semi-stagnant pond, July 2, 1925, *Frontonia* with very few zoöchlorellæ were abundant.

Fig. 2. Typical specimen from a collection in which the *Frontonia* contained but few zoöchlorellæ.

Slides from the collection showed the average specimen to contain from fifty to one hundred zoöchlorellæ (Fig. 2). The

[1] Make a thin smear of Mayer's egg albumen the size of a dime on a clean slide. Place the ciliates to be fixed on the smear in a drop of water just large enough to make a thin layer over the albumen. Invert the slide over the mouth of a small bottle containing a mixture of equal parts of 95 per cent. alcohol, glacial acetic acid and formalin. Let the slide remain in this position 30 seconds to a minute. Remove the slide and slant it back and forth until the film of water has almost disappeared. Wash gently in water and stain.

collection was set aside and remained untouched until August 1. On this date the zoöchlorellæ in twelve specimens examined were innumerable (Fig. 3).

On June 8, 1926, eight cultures, containing twelve specimens per culture, of *Frontonia* harboring relatively few zoöchlorellæ, were started in fresh water to which a small amount of detritis

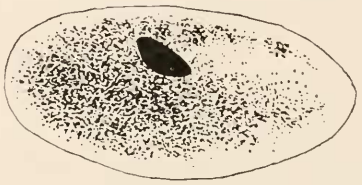

FIG. 3. Showing increase of zoöchlorellæ in *Frontonia* after the same collection (Fig. 2) had remained in the laboratory for thirty days.

from the collecting dish was added. These cultures remained in the laboratory with abundant light, but never in direct sunlight, until July 8, without adding more fresh spring water. At this time slides were made from each culture. The zoöchlorellæ in 95 per cent. of the animals were innumerable. Checks which corroborate these findings were made with cultures started June 10 and 12.

From these results the conclusion, that the number of zoöchlorellæ in *Frontonia leucas* may be increased by an increase in stagnation and putrefaction of the medium in which they live, seems logical.

INFLUENCE OF OSMOTIC PRESSURE.

In an effort to explain, beyond the simple statement of the difference between stagnant and fresh water, these variations in the number of zoöchlorellæ inhabiting each host, cultures with various osmotic pressures were made as follows: Three cultures using distilled water, three cultures using fresh spring water, three cultures of one per cent. dextrose in spring water. Into each of these cultures were placed six specimens in which the zoöchlorellæ were numerous, and six with no zoöchlorellæ. It was found that upon being put into distilled water the organisms with zoöchlorellæ promptly disintegrated. Ejection of zoö-

chlorellæ, desmids, and diatoms preceding death only by a few moments. The organisms which contained no zoöchlorellæ often lived several hours. In fresh spring water specimens with zoöchlorellæ lived from five to eight days. Specimens without zoöchlorellæ lived indefinitely. In one per cent. dextrose specimens with zoöchlorellæ lived eight days or more. Specimens without zoöchlorellæ averaged two days. It was found also that if the cultures in one per cent. dextrose were placed in fresh media daily, specimens with zoöchlorellæ lived indefinitely, while those without lived four or five days.

Experiments begun June 24, 1926, showed that *Frontonia* with abundant zoöchlorellæ may be induced to live several days in a three per cent. solution of dextrose by beginning at one half per cent., changing the medium daily, and increasing the concentration one half per cent. every fourth day. But under no condition have specimens without zoöchlorellæ been induced to live more than a few hours in a medium exceeding one per cent.

In two cultures containing one per cent. dextrose vegetative reproduction was observed in *Frontonia* harboring zoöchlorellæ where fermentation had been going on three and four days. *Frontonia* without zoöchlorellæ would not live in a medium of this kind.

These observations suggest that specimens harboring zoöchlorellæ are more resistant to media of greater osmotic pressure than are free specimens.

Hydrogen Ion Concentration.

It has been observed that as fermentation progresses in dextrose cultures the hydrogen ion concentration increases; *e.g.*, a one per cent. solution with a pH value of 7.2 made July 18 showed, 24 hours later, a pH of 7.1. After 48 hours the pH was 7.0, after 72 hours 6.9, and on the fourth day it had become 6.7. Observations made on one, two, and three per cent. solutions showed similar increases in hydrogen ion concentration during fermentation.

The hydrogen ion concentration of media from natural collections was then studied. Data gathered from this study are given in Table I.

Table I.

Showing Hydrogen Ion Concentration of Three Collecting Pools over an Extended Period of Time.

Date	August, 1925.							June and July, 1926.							
	1	4	7	10	13	15	17	12	15	18	21	24	27	30	8
A...	7.4	7.4	7.3	7.3	7.4	7.5	7.5	7.3	7.3	7.3	7.4	7.4	7.4	7.5	7.5
B...	7.4	7.3	7.3	7.3	7.4	7.4	7.4	7.3	7.3	7.2	7.2	7.3	7.3	7.4	7.4
C...	7.2	7.2	7.1	7.1	7.1	7.2	7.2	7.1	7.1	7.0	7.0	7.2	7.2	7.3	7.4

A from pool containing *Frontonia* with no zoöchlorellæ.

B with relatively few.

C in which the zoöchlorellæ were innumerable.

Beginning June 20th there were increasing rains until July 10. On July 12 collections were taken from all pools where *Frontonia* had previously been found and a uniform scarcity of zoöchlorellæ was observed.

As stated above, when specimens containing relatively few zoöchlorellæ are placed in cultures and left undisturbed, so that the culture medium becomes somewhat stagnant, the zoöchlorellæ increase very noticeably. Also that when specimens containing numerous zoöchlorellæ are changed from their original culture to a medium of fresh spring water, the zoöchlorellæ decrease very noticeably. There is correlated with this increase and decrease in the number of zoöchlorellæ, an increase and decrease in the hydrogen ions. The following examples are characteristic of the finding in this respect.

A collection in which the *Frontonia* showed relatively few zoöchlorellæ had a pH value of 7.3. This culture remained in the laboratory and the hydrogen ion concentration was taken every other day until the specimens harbored innumerable zoöchlorellæ, thirty days. Data thus obtained are given in Table II.

Table II.

Showing Variations in pH Value of the Medium as the Number of Zoöchlorellæ Increased.

	June and July, 1926.													
Date.....	10	12	14	16	18	21	24	27	30	3	6	10	14	18
pH.....	7.3	7.3	7.2	7.0	6.9	6.9	6.7	6.7	6.8	6.8	6.9	7.0	7.0	7.1

A collection in which the *Frontonia* showed innumerable zoöchlorellæ had a pH value of 6.8. This culture remained in the laboratory and a change from the original culture medium to fresh spring water was made gradually. The hydrogen ion concentration was taken every other day until the specimens harbored few, and in some cases no zoöchlorellæ. Data thus obtained are given in Table III.

Table III.

Showing Variations in pH Value of the Medium as the Number of Zoöchlorellæ Decreased.

June and July, 1926.

Date.....	8	10	12	14	16	18	20	22	25	28	30	4	7	10
pH.....	6.8	6.8	6.9	6.9	6.9	7.0	7.0	7.0	7.1	7.2	7.2	7.2	7.3	7.3

Discussion.

An apparently significant fact observed during the course of these experiments is: That *Frontonia* with abundant zoöchlorellæ refuse to live in media of clear, fresh water. They seem to require a stagnant, even putrid, medium containing detritis, which serves as a place of hiding, much as do *Spirostomum ambiguum*. Indeed these two protozoans have a common habitat in every instance within the experience of the writer. But *Frontonia* without zoöchlorellæ have been found in great abundance in fresh running streams.

It will be noticed from the foregoing investigations that a host may experience either an increase or a decrease in the number of its zoöchlorellæ, but that no statement is made concerning their ability to regain them after having been completely freed. Regarding this subject we may say that repeated efforts to induce *Frontonia* with no zoöchlorellæ to acquire them were unsuccessful. Dangeard says of the zoöchlorellæ of *Paramecium bursaria* that they do not live long when freed in the medium of their host. This suggested the possibility of inducing *Frontonia* without zoöchlorellæ to appropriate them directly from the body of a crushed specimen. Efforts to demonstrate this, however, were unsuccessful.

Another feature that seems worthy of further investigation may be observed in the camera lucida drawings given in Fig. 1; *e.g.*, the evident decrease in the size of the host as the zoöchlorellæ disappear. Minchin quotes Popoff as observing these variations in size of *Frontonia leucas* and crediting them to a difference in temperature of the media in which the *Frontonia* live.

The experiments of this paper were conducted in a laboratory where both large and small, infected and uninfected *Frontonia* were exposed to the same conditions of temperature. Yet differences of as much as 100 microns have been found in specimens taken from the same culture, and these variations have persisted throughout the study. Therefore it seems unlikely that temperature could have had any part in this variation.

Of fifty specimens, without zoöchlorellæ, measured, the average length was 225 microns, while the nucleus averaged 16 microns in length. Of fifty specimens, with innumerable zoöchlorellæ, measured, the average length was 310 microns and the nucleus averaged 18 microns in length.

These observations tend to demonstrate that a mass relation exists between cytoplasm and nucleus. Similar nucleoplasmic relations have been demonstrated in different species of *Arcella* by Hegner (1920) and Reynolds (1923).

Lipska (1910) found that when placed in the dark, *Paramecium caudatum* with zoöchlorellæ would live eight days or more, that *Paramecium* without zoöchlorellæ died between the second and fourth days, and concluded that the zoöchlorellæ were responsible for the greater resistance shown by the *Paramecium* with zoöchlorellæ, in that they furnished a supply of oxygen.

The following experiment was carried out with *Frontonia leucas:* Specimens with zoöchlorellæ were placed in darkness, in their original culture medium, and exposed to the same conditions of temperature as before, but no appreciable difference was noticed in their death rate as compared with those in the light. This, however, does not preclude the possibility of the host obtaining oxygen from the zoöchlorellæ, because in darkness, where photosynthesis is impossible, zoöchlorellæ cannot give off oxygen.

In his work on the zoöchlorellæ of *Paramecium bursaria*, Dangeard found that starch granules were abundant in the zoöchlorellæ and it was his belief that the host appropriated these starch granules as food, though he was unable to demonstrate this.

In this work on the zoöchlorellæ of *Frontonia*, starch granules were found to be abundant in the zoöchlorellæ, but efforts to show that they were ever taken into the food vacuoles of the host have thus far been unsuccessful.

Summary.

1. *Frontonia* with zoöchlorellæ require a medium of greater stagnation and putrefaction than *Frontonia* without zoöchlorellæ.

2. *Frontonia* may be freed from their zoöchlorellæ by a gradual transference from their natural habitat to a medium of fresh spring water.

3. The number of zoöchlorellæ in *Frontonia* may be increased by increasing the degree of stagnation and putrefaction of the medium in which they live.

4. *Frontonia* harboring zoöchlorellæ are more resistant to media of greater osmotic pressure than are free specimens.

5. The difference between the hydrogen ion concentration of media in which *Frontonia* with innumerable zoöchlorellæ are found and media in which *Frontonia* with no zoöchlorellæ are found, averages 0.3.

6. With an increase in the number of zoöchlorellæ there is a corresponding increase in the hydrogen ions, and conversely.

7. The ability of *Frontonia* harboring zoöchlorellæ to live in a greater concentration of dextrose than *Frontonia* without zoöchlorellæ is correlated with a greater hydrogen ion concentration caused by fermentation of the dextrose.

8. Efforts to induce *Frontonia* without zoöchlorellæ to gain them were unsuccessful.

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